Shigeo MASUYAMA*: The gametophyte of *Pleurosoriopsis makinoi* (Maxim.) Fomin

益山樹生*: カラクサシダの配偶体

Pleurosoriopsis makinoi (Maxim.) Fomin, the sole species of the genus, is a small fern growing among mosses on rocks in wet gullies in Japan, Korea, north China and the Amur region of USSR. It was treated as an asplenioid fern by Christensen (1938) and Ching (1940), but later assigned to the Pteridaceae by Copeland (1947). More recently, Holttum (in Willis, 1966) kept it in the gymnogrammoid group, though he (1949) had tentatively treated it as an asplenioid fern. In these discussions, however, little attention has been paid to the gametophyte characters. In this paper, an account will be given to the gametophyte morphology of this species and a discussion will be made for the relationships between this species and various species of other genera.

Materials and methods The fertile fronds of *P. makinoi* were collected on Mt. Jii-dake, Toyama Pref. in November 1971 and on Mt. Iide, Niigata Pref. in November 1972. The gametophytes growing in the field were collected on Mt. Ojika, Fukushima Pref. in May 1974 and they were compared with cultivated ones. The fertile fronds were washed well with running tap water and kept within clean drag-papers to allow shedding their spores. The spores were sown on an inorganic nutrient medium in small Petri-dishes. The medium was four-fold diluted Knop's solution supplemented with 2 cc of soil extract (supernatent of autoclaved solution containing 40% soil) per liter and solidified with 1% agar. For the cultivation of gametophytes, the Petri-dishes were placed in a growth chamber (23±1°C and about 2000 lux, but illuminated for 12 hours a day with white fluorescent tubes). Mature gametophytes were fixed in Craf I and sectioned following the usual paraffin procedure. Some mature gametophytes were transferred on soil in small clay pots kept in a greenhouse for a long-term culture. Camera lucida drawings were made of sections and external features of the gametophytes.

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Observation The spores are bilateral with slightly scabrate surface. They are chlorophyllous (Fig. 1). The first and, sometimes, the second divisions took place within the spore coat before they were shed from sporangia (Fig. 2). The first wall was parallel to the polar axis of the spore and the resulting two cells were almost equal in size (Fig. 3). These two cells became more or less globose and were divided into two cells respectively by the second wall parallel to the first wall (Figs. 4–6). Subsequent divisions took place in one of the terminal cells to form a filament of gametophyte (Figs. 9–11).

When the filaments reached four- to six-cell stage (in about two weeks after the spore-sowing), the first rhizoid was formed on one of the terminal cells or the cell next to one of the terminal cells of the filament (Figs. 7–8). It was hyaline at first, turning into pale brown later. The second rhizoid was formed on the cell next to the cell with the first rhizoid (Fig. 9), but sometimes on the

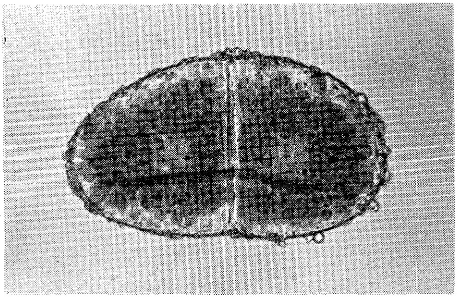
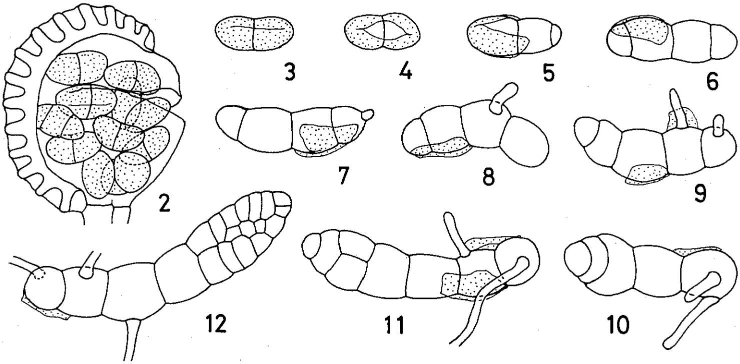


Fig. 1. A spore containing chloroplasts, ×450.



Figs. 2-12. Germination of spores and early stages of development of gametophytes, ×100.

same cell that formed the first rhizoid (Fig. 10).

In about one month after the sowing, when the filaments were composed of six cells or more, they started forming the primary plates of gametophytes (Fig. 11); the first longitudinal division occurred in a cell which was located two or three cells behind the tip of the filament, and the subsequent vertical and longitudinal cell divisions followed it. About a half month later, oblique divisions occurred in the apex of the plate and a wedge-shaped cell was formed (Fig. 12).

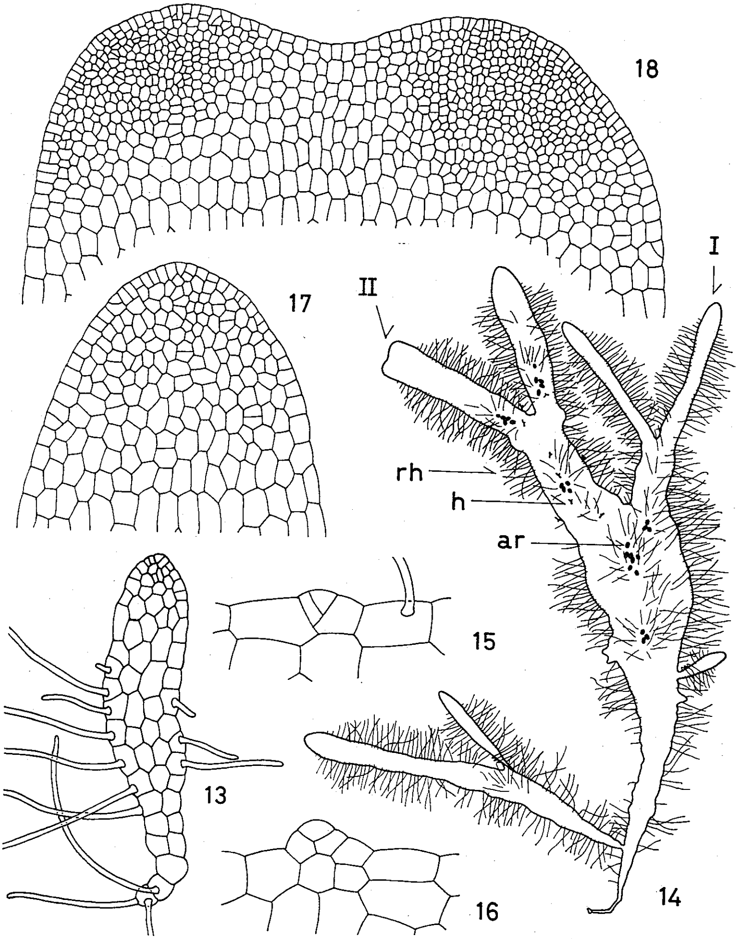
In about three months after the sowing, when the gametophytes were spatulate, small protrusions arose from the cell at the lower margin of the plate to form brownish marginal rhizoids (Fig. 13).

In about ten months after the sowing, the gametophytes attained maturity to produce sex organs. They were thalloid and formed several secondary plates on the primary plate (Fig. 14). The secondary plates developed from a wedge-shaped cell at the margin of the primary plate (Figs. 15–16) or they were formed by the meristem in the apex of the primary plate (Fig. 18). At the tips, these secondary plates had meristems which were not clearly defined (Fig. 17). The primary and secondary plates formed cushions, sex organs and hairs.

The cushion was formed on the median plane of the plate. It was only two or three cells thick, narrow and discontinuous. Archegonia and rhizoids were formed on its ventral side.

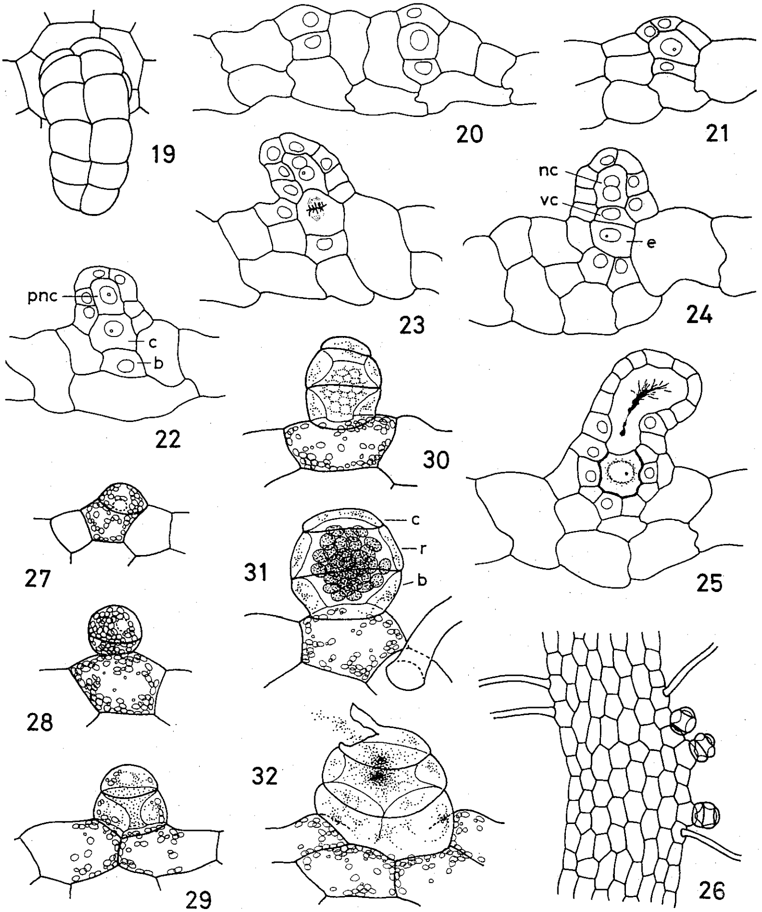
Archegonia were formed in the cushion at some distance from the apex of the plate. Their developmental sequence is shown in Figs. 20–25. The division of the central cell into a ventral canal cell and an egg cell occurred after the neck canal cell had reached binuclear stage (Figs. 22–24). In mature archegonia, the neck was composed of four rows of cells and each row consisted of longitudinally arranged six to seven cells (Fig. 19).

Antheridia were formed on the margin of only the secondary plate after a number of archegonia were produced on the cushions of the primary and secondary plates (Fig. 26). Their developmental sequence is shown in Figs. 27–32. In mature antheridia, the wall consisted of three cells: a funnel-shaped basal cell, a ring cell and a cap cell (Fig. 31). The sperms were released through a pore formed on the lateral side of the cap cell (Fig. 32).



Figs. 13-18. Young and mature gametophytes. 13. Young gametophyte, ×40. 14. Mature gametophyte, ×5. *ar*, archegonium; *h*, hair; *rh*, rhizoid. 15-16. Early stages in development of branch, ×95. 17. Apical part of the plate I shown in Fig. 14, ×80. 18. Apical part of the plate II shown in Fig. 14, ×80.

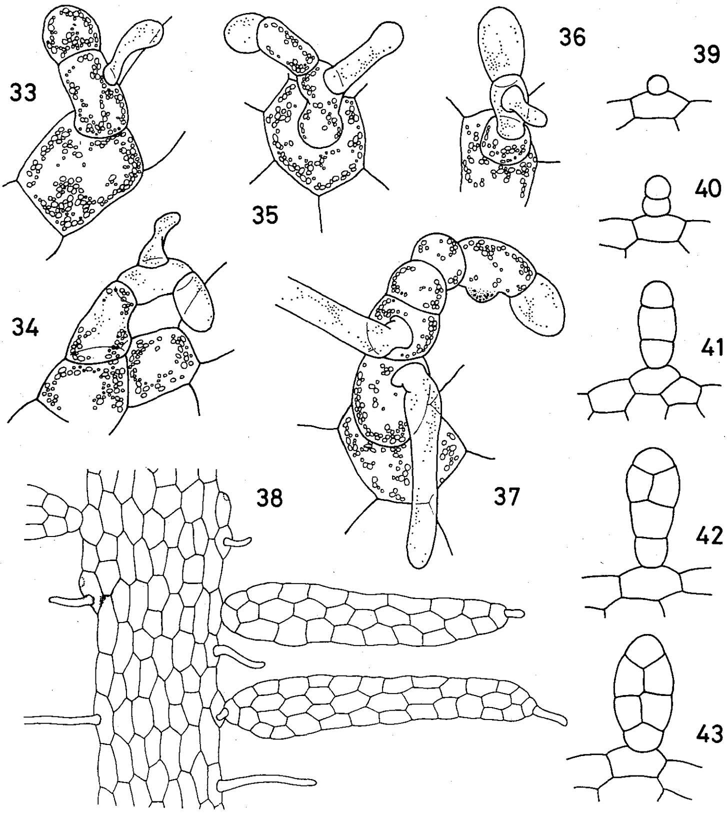
The hairs were formed on the cushions or less frequently on the margins of some well-developed gametophytes (Fig. 14). They were usually composed of two or three cells and had a unicellular hyaline appendage (Figs. 33-36). Sometimes, however, four- to six-celled hairs with two or



Figs. 19-32. Sex organs. 19. Mature archegonium, ×210. 20-25. Stages of development of archegonia, ×210. *pnc*, primary neck canal cell; *c*, central cell; *b*, basal cell; *nc*, neck canal cell; *vc*, ventral canal cell; *e*, egg cell. 26. Antheridia on margin of plate, ×45. 27-32. Stages of development of antheridia, ×210. *c*, cap cell; *r*, ring cell; *b*, basal cell.

three prolonged hyaline appandages were observed (Fig. 37). The cells of hairs were somewhat inflated and contained chloroplasts, though one or two distal cells lacked chloroplasts in mature stages. The terminal cell of hairs appeared to be a glandular cell, but no secretion was observed.

The gametophytes more than about one and half years old, which had been transferred on soil, had numerous secondary plates. These plates were far more slender than the primary one and produced many gemmae on their margins (Fig. 38). The gemma originated from a small protrusion on a marginal cell and developed into a narrowly elliptic plate by subsequent cell divisions, as illustrated in Figs. 39-43. The terminal cell and, sometimes, the basal cell of gemma bore a papilla (Fig. 38). The gemmae were easily detached from the plates and developed into independent gametophytes.



Figs. 33-43. Hairs and gemmae. 33-37. Hairs on filament (33), margin (34) and ventral surface (35-37), ×210. 38. Gemmae on margin of plate, ×50. 39-43. Stages of development of gemmae, ×100.

Morphological characters mentioned above have been also observed in gametophytes growing in the field (collected on Mt. Ojika, Fukushima Pref.) and no notable difference has been found between gametophytes growing in the field and cultivated in the laboratory. It may be noted that cultivated gametophytes transferred on soil are still continuing to form new plates and gemmae in the laboratory, even though it has been nearly three years since the sowing.

Discussion The formation of the first wall perpendicular to the polar axis of the spore is most commonly found in spore germination of ferns. However, in *Pleurosoriopsis*, the first wall is parallel to the polar axis of the spore. Nayar and Kaur (1971) are of opinion that this type of spore germination is characteristic of the gleichenioid-polypodioid group. Many species of the Gleicheniaceae and Polypodiaceae* actually show this type, as reported by Stokey (1950), Nayar (1967) and so on. However, some species of the Polypodiaceae do not follow this type, as pointed out by Nayar and Kaur (1971). Furthermore, this type of spore germination has been observed also in some species of several other groups: *Lomariopsis rosii* in Aspidiaceae (Atkinson, 1973), for example. Therefore, the formation of the parallel wall does not seem to be a common character in certain groups which are phyletically related each other. It is noteworthy that, in most species which follow this type, the first division of spore takes place while the spore is kept in the spore coat, a feature quite rare in ferns.

The spatula-shaped gametophyte with obscure terminal meristem is relatively common in species of Hymenophyllaceae, Polypodiaceae and Vittariaceae. They are, for example, *Hymenophyllum acanthoides* (Stokey, 1940), *Loxogramme salicifolia* (Momose, 1967), *Vittaria lineata* (Wagner & Sharp, 1963) etc. However, gametophyte of this type has been observed in several species of other unrelated families such as *Anogramma leptophylla* in Pteridaceae (Goebel, 1877), *Arthropteris tenella* in Davalliaceae (Atkinson, 1973) and *Lomariopsis hederacea* in Aspidiaceae (Atkinson, 1973). These species are mostly rupicolous or epiphytic, as in the case of *Pleurosoriopsis*. The spatula-shaped gametophyte with obscure terminal meristem, therefore, seems to be associated with such habit.

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* In this discussion, genera and families are used in the sense of Copeland (1947).

Although multicellular hairs are widely distributed in the fern gametophytes (cf. Stokey, 1960; Nayar & Kaur, 1973), hairs similar to those of *Pleurosoriopsis makinoi* are found only in some species of the Davalliaceae, Aspleniaceae, and Polypodiaceae. Among species of the Davalliaceae and the Aspleniaceae, some species such as *Nephrolepis cordifolia* and *Ceterach officinarum* form three- to seven-celled hairs (Stokey, 1960). As in hairs of *Pleurosoriopsis*, in addition, these hairs are composed of more or less inflated cells, which contain chloroplasts except for in cases of a few distal cells. However, they are usually simple and have no appendage, while those of *Pleurosoriopsis* have appendages. Multicellular hairs of *Arthropteris tenella* (Davalliaceae) resemble those of *Pleurosoriopsis* in having some appendages. However, these hairs are appressed to the margin of gametophytes and entirely lack chloroplast (Stokey, 1960). These multicellular hairs found in the Davalliaceae and the Aspleniaceae, therefore, are different from those of *Pleurosoriopsis* in certain aspects. In contrast, many species of the Polypodiaceae form multicellular hairs which are quite similar to those of *Pleurosoriopsis*. They are some species of *Platycerium* (Stokey & Atkinson, 1954), *Pyrrosia* and other genera (Stokey, 1960), *Polypodium* and other genera (Nayar, 1962), *Microsorium* and other genera (Momose, 1967, 1968). As in *Pleurosoriopsis*, hairs of these species are composed of more or less inflated three to seven cells, which contain chloroplasts except for in cases of a few distal cells, and have one or more hyaline appendages. The similarity in morphological features of hairs mentioned here seems to indicate the close relationship between *Pleurosoriopsis makinoi* and species of the Polypodiaceae.

The gemma formation of gametophytes has been known in the following species: *Vandenboschia auriculata* and some species of the Hymenophyllaceae (Stokey, 1948; Stone, 1958; Farrar & Wagner, 1968; Yoroi, 1972), *Xiphopteris serrulata* and some species of the Polypodiaceae (Stokey & Atkinson, 1958; Nayar, 1963), and *Vittaria lineata* of the Vittariaceae (Farrar, 1967). Gemmae of these species are very variable in shape and they are also different from those of *Pleurosoriopsis*. It is noteworthy that these species, including *Pleurosoriopsis makinoi*, are all rupicolous or epiphytic. In connection with this, it should be noted that gemmiferous gametophytes are usually found on dump rocks, where the light intensity is very low (Farrar,

1967) and various rupicolous or epiphytic ferns grow well. The gemma formation, therefore, seems to be a character associated with the rupicolous or epiphytic habit of these species.

Other characters of the gametophyte of *Pleurosoriopsis makinoi*, such as sex organs, are commonly found in other species of the Leptosporangiate ferns.

The gametophyte of *Pleurosoriopsis makinoi* shows several features which are rarely found in fern gametophytes. As discussed above, most of these features seem to be associated with the rupicolous habit. However, morphological features of hairs of *P. makinoi* show considerable similarities to those of the Polypodiaceae. Therefore, *P. makinoi* seems to be most closely related to the Polypodiaceae at least in regards to the gametophyte morphology.

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カラクサシダ（*Pleurosoriopsis makinoi*）の配偶体の培養と観察を行ない次の知見を得た。

胞子は両面体。早くから葉緑体をもち胞子のう内で壁内発芽をする。第一回の分裂で胞子は背腹軸に平行な膜によって二等分される。初生仮根は 4〜6 細胞期に形成される。発達した配偶体はハート形にならず，不定形。しばしば分岐する。造精器，造卵器，毛，無性芽が形成される。造精器，造卵器はいずれも薄のうシダ類に一般的な型。毛は多細胞分岐毛で，先端部の 1〜2 細胞と枝細胞には葉緑体が見られない。無性芽は平板で，長楕円形。

これらの形質のうち，多細胞分岐毛はウラボシ科に見られるものと強い類似性を示し，カラクサシダとウラボシ科の類縁性を示唆しているように思われる。他の特徴的な形質，胞子の発芽様式，配偶体の全形，無性芽からはカラクサシダと他群との類縁性を推定することは困難である。

□Foster, A.S. & Gifford, E.M.: **Comparative Morphology of Vascular Plants,** 2nd edition. 751 pp. 1974. Freeman and Company. $17.　本書は 1959 年に出版され比較形態学の教科書として好評だった初版の改訂版で，全体にわたってかなり大幅な内容の変更が行われている。比較的簡単に扱われていた被子植物では花粉・胚発生等の記述も充実し，初版では言及されなかった花序等についてもかなり詳しい説明が加わった。記述は従来通り全分類群に首尾一貫した方針が採られ，全体としてよくバランスがとれている。ただし最近進化，系統に関係して多くの問題提起のあった Cycadeoidea や古生マツバラン類は幾分簡単に扱われているように思われる。後者については，Banks に従って Rhyniopsida, Zosterophyllopsida, Trimerophytopsida という独立の綱に分ける思い切った扱いをしている。反面一連の Rhynie 植物研究から Lemoigne が 1968 年に発表した Banks 説と対照的な分類には一言もふれていない。また増加の著しいデヴォン紀以前の維管束植物化石の吟味もおざなりである等多少の物足りなさを覚えるところもある。　（大場秀章）